

WHAT IS CLAIMED IS:

1. A method for identifying a compound that regulates the activity of autoinducer-2 comprising:

- (a) contacting autoinducer-2 with the compound;
- (b) measuring the activity of autoinducer-2 in the presence of the compound and comparing the activity of autoinducer-2 obtained in the presence of the compound to the activity of autoinducer-2 obtained in the absence of the compound; and
- (c) identifying a compound that regulates the activity of autoinducer-2.

2. The method of claim 1, wherein the autoinducer-2 is 4-hydroxy-5-methyl-2H-furan-3-one.

3. The method of claim 1, wherein the contacting is *in vivo*.

4. The method of claim 1, wherein the contacting is *in vitro*.

5. The method of claim 1, wherein the regulation is by increasing the activity of autoinducer-2.

6. The method of claim 1, wherein the regulation is by decreasing the activity of autoinducer-2.

7. The method of claim 1, wherein the compound is a polypeptide.

8. The method of claim 1, wherein the compound is a small molecule.

9. The method of claim 1, wherein the compound is a nucleic acid.

10. A method for identifying an autoinducer-2 analog that regulates the activity of autoinducer-2, comprising:

(a) contacting a bacterial cell, or extract thereof, comprising biosynthetic pathways which will produce a detectable amount of light in response to autoinducer-2, with the autoinducer analog;

(b) comparing the amount of light produced by the bacterial cell, or extract thereof, in the presence of the autoinducer-2 with the amount produced in the presence of the autoinducer-2 and the autoinducer-2 analog, wherein a change in the production of light is indicative of an autoinducer-2 analog that regulates the activity of autoinducer-2.

11. The method of claim 10, wherein the autoinducer-2 is endogenous autoinducer-2.

12. The method of claim 10, wherein the autoinducer-2 is synthesized in a bacterial cell or by an extract thereof.

13. The method of claim 10, wherein the autoinducer-2 is exogenous autoinducer-2.

14. The method of claim 10, wherein the contacting is *in vitro*.

15. The method of claim 10, wherein the contacting is *in vivo*.

16. The method of claim 10, further comprising contacting the bacterial cell, or extract thereof, with autoinducer-2.

17. The method of claim 10, wherein the regulation is by inhibition of autoinducer-2 activity.

18. The method of claim 10, wherein the regulation is by enhancement of autoinducer-2 activity.

19. The method of claim 10, wherein the analog comprises a ribose derivative.

20. The method of claim 10, wherein the bacterial cell, or extract thereof, further comprises at least one distinct alteration in a gene locus that participates in an autoinducer pathway, wherein the alteration inhibits the production or detection of an autoinducer.

21. The method of claim 20, wherein the alteration in a gene locus comprises an alteration in the LuxS gene.

22. The method of claim 20, wherein the alteration in a gene locus inhibits production of autoinducer-2.

23. The method of claim 20, wherein the alteration in a gene locus comprises an alteration in the LuxN gene.

24. The method of claim 20, wherein the alteration in a gene locus inhibits detection of autoinducer-1.

25. The method of claim 20, wherein the alteration is in the LuxN and LuxS loci.

26. The method of claim 20, wherein the bacterial cell is *V. harveyi* strain MM32.

27. A method for identifying a compound that regulates the production or activity of autoinducer-2, comprising:

contacting a bacterial cell that produces autoinducer-2 with the compound,  
and

determining whether autoinducer-2 activity is present in the bacterial cell.

28. The method of claim 27, wherein autoinducer-2 activity is determined by detecting the inhibition of autoinducer-2 production.

29. The method of claim 28, wherein autoinducer-2 activity is determined by detecting a signal produced in the presence of autoinducer-2.

30. The method of claim 29, wherein the method detects an antagonist of autoinducer-2.

31. The method of claim 30, wherein the method detects a change in luminescence from a reporter bacterial strain.

32. The method of claim 31, wherein the bacterial strain is of the genus *Vibrio*.

33. The method of claim 32, wherein the bacterial strain is of the species *Vibrio harveyi*.

34. The method of claim 33, wherein the bacterial strain is *Vibrio harveyi* BB170.

35. The method of claim 33, wherein the bacterial strain is *Vibrio harveyi* MM32.

36. A method for detecting an autoinducer-associated bacterial biomarker comprising;

(a) contacting at least one bacterial cell with an autoinducer molecule under conditions and for such time as to promote induction of a bacterial biomarker;  
and

(b) detecting the bacterial biomarker.

37. The method of claim 36, wherein the autoinducer is autoinducer-2.

38. The method of claim 36, wherein the autoinducer-2 is 4-hydroxy-5-methyl-2H-furan-3-one.

39. A method for detecting an autoinducer-associated biomarker comprising:

(a) contacting at least one cell with an autoinducer molecule under conditions and for such time as to promote induction of a biomarker; and

(b) detecting the biomarker.

40. The method of claim 39, wherein the autoinducer is autoinducer-2.

41. The method of claim 40, wherein the autoinducer-2 is 4-hydroxy-5-methyl-2H-furan-3-one.

42. A method for identifying a compound that affects autoinducer-2 binding to an autoinducer-2 receptor, comprising:

(a) contacting autoinducer-2 and the autoinducer-2 receptor with the compound to allow autoinducer-2 binding to the autoinducer-2 receptor;

(b) contacting the product of a) with a cell, or cell extract, comprising biosynthetic pathways that produce light in response to autoinducer-2 binding to the autoinducer-2 receptor; and

(c) measuring the effect of the compound on light production,

wherein a change in light production in the presence of the compound, compared to light production in the absence of the compound, identifies the compound as one that affects binding of autoinducer-2 to the autoinducer-2 receptor.

43. The method of claim 42, wherein the compound is selected from the group consisting of competitive inhibitors and suicide inhibitors.

44. The method of claim 42, wherein the autoinducer-2 receptor is selected from the group consisting of luxP and luxN.

45. The method of claim 42, wherein the autoinducer-2 is allowed to form a complex with the autoinducer-2 receptor in the absence of the compound.

46. The method of claim 42, wherein the autoinducer-2/autoinducer-2 receptor complex is bound to a solid support medium.

47. The method of claim 46 wherein the solid support medium is selected from the group consisting of a column matrix and a microtiter dish well.

48. The method of claim 47, wherein the autoinducer-2/autoinducer-2 receptor complex is bound to a solid support medium through a linkage selected from the group consisting of amide, ester, and ether.

49. A method for producing autoinducer-2 comprising:

(a) contacting S-adenosylhomocysteine (SAH) with a 5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase (pfs) protein whereby S-adenosylhomocysteine undergoes conversion to S-ribosylhomocysteine;

(b) contacting the S-ribosylhomocysteine from (a) with a LuxS protein whereby S-ribosylhomocysteine undergoes conversion to autoinducer-2.

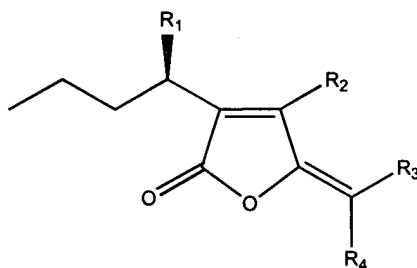
50. The method of claim 49, wherein the autoinducer-2 is 4-hydroxy-5-methyl-2H-furan-3-one.

51. Isolated autoinducer-2 prepared in accordance with the method of claim 49.

52. An antibiotic composition comprising an antibiotic and an inhibitor of the quorum-sensing pathway of a microorganism.

53. The antibiotic composition of claim 52, wherein the inhibitor inhibits the AI-1 quorum-sensing pathway.

54. The antibiotic composition of claim 52, wherein the inhibitor is a halogenated 2(5H) furanone of the structure:



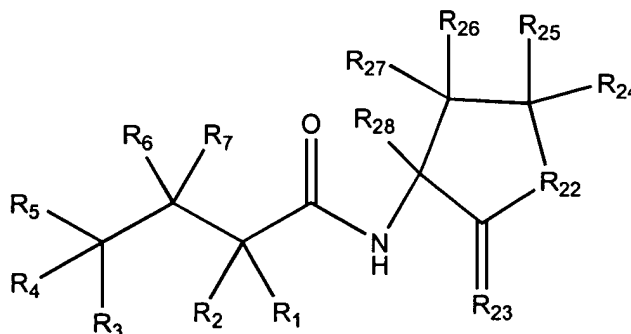
wherein R<sub>1</sub> is a hydrogen atom, an acetoxy group, or a hydroxy group;

R<sub>2</sub> is a hydrogen atom or a bromo group;

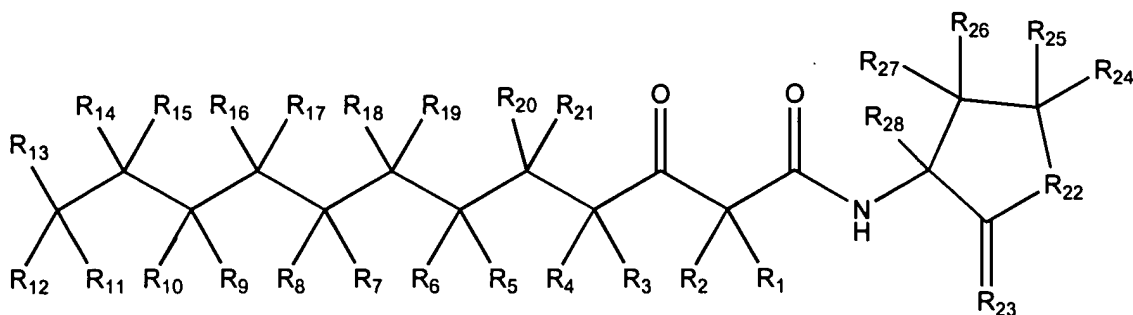
R<sub>3</sub> is a hydrogen atom or a bromo group; and

R<sub>4</sub> is a bromo or iodo group.

55. The antibiotic composition of claim 53, wherein the inhibitor that inhibits the AI-1 quorum-sensing pathway is a modified N-butyryl-L-homoserine compound of the structure:



or a modified N-(3-oxododecanoyl)-L-homoserine lactone compound of the structure:



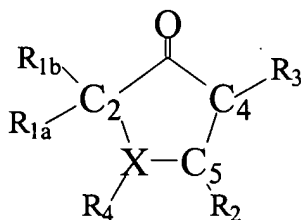
wherein in the above structures  $R_1$  through  $R_{21}$  are each individually selected from the group consisting of a  $C_1$  to  $C_4$  alkyl group, a hydrogen atom, a hydroxy group, an amino group, and a thiol group;

$R_{22}$  and  $R_{23}$  are each individually an oxygen or a sulfur atom; and

$R_{24}$  -  $R_{28}$  are each individually a hydrogen atom or a halogen atom.

56. The antibiotic composition of claim 52, wherein the inhibitor inhibits the AI-2 quorum-sensing pathway.

57. The antibiotic composition of claim 56, wherein the inhibitor that inhibits the AI-2 quorum-sensing pathway comprises the structure:



wherein X is oxygen, sulfur or nitrogen;

$R_{1a}$  is hydrogen, hydroxy, alkyl, acyl, amido, hydroxyl, amino, thio, or aryl;

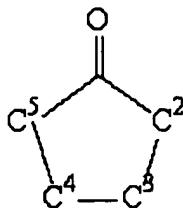
$R_{1b}$  is hydrogen, hydroxy, alkyl, acyl, amido, hydroxyl, amino, mercapto, thio, or aryl, or  $R_{1a}$  and  $R_{1b}$  can together form a double bond;

$R_2$  is hydrogen, alkyl, or halogen;

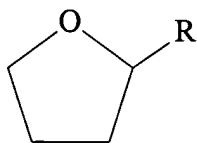
$R_3$  is hydrogen, alkyl, acyl, amido, hydroxyl, amino, thio, or aryl;

$R_4$  is hydrogen, if X is nitrogen, or is absent if X is oxygen or sulfur; and

wherein  $C_4$  and  $C_5$  can optionally be further joined by a double bond; or comprises the structure



wherein C<sup>2</sup> is additionally bonded to at least one substituent selected from hydrogen, hydroxyl, C<sub>1-5</sub> alkyl, C<sub>2-5</sub> alkenyl, C<sub>2-5</sub> alkanoyl, C<sub>2-5</sub> alkanoyloxy, heteroaryl, or forms a double bond with an oxygen atom or C<sup>3</sup>; wherein C<sup>3</sup> is additionally bonded to at least one substituent selected from hydrogen, hydroxyl, C<sub>1-5</sub> alkyl, C<sub>2-5</sub> alkenyl, C<sub>2-5</sub> alkanoyl, C<sub>2-5</sub> alkanoyloxy, or forms a double bond with an oxygen atom or C<sup>2</sup>; wherein C<sup>4</sup> is additionally bonded to at least one substituent selected from hydrogen, C<sub>1-5</sub> alkyl, C<sub>2-5</sub> alkanoyl, C<sub>2-5</sub> alkanoyloxy, or forms a double bond with an oxygen atom or C<sup>5</sup>; and wherein C<sup>5</sup> is additionally bonded to at least one substituent selected from hydrogen, C<sub>1-5</sub> alkyl, C<sub>2-5</sub> alkanoyl, C<sub>2-5</sub> alkanoyloxy, or forms a double bond with an oxygen atom or C<sup>4</sup>, wherein at least one of C<sup>2</sup>, C<sup>3</sup>, C<sup>4</sup> or C<sup>5</sup> is bonded to a substituent selected from hydroxyl, C<sub>1-5</sub> alkyl, C<sub>2-5</sub> alkenyl, C<sub>2-5</sub> alkanoyl, and heteroaryl, and wherein at most one carbon-carbon double-bond is present in the ring; or comprises the structure



wherein R is a C<sub>1-5</sub> alkoxy group.

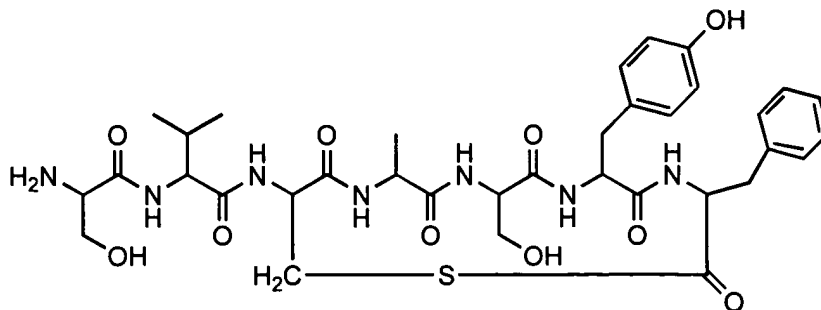
58. The antibiotic composition of claim 57, wherein the inhibitor is 5-methyl-2-ethyl-4-hydroxy-3(2H)-furanone.

59. The antibiotic composition of claim 57, wherein the inhibitor is 2,5 dimethyl-4-hydroxy-3(2H)-furanone.

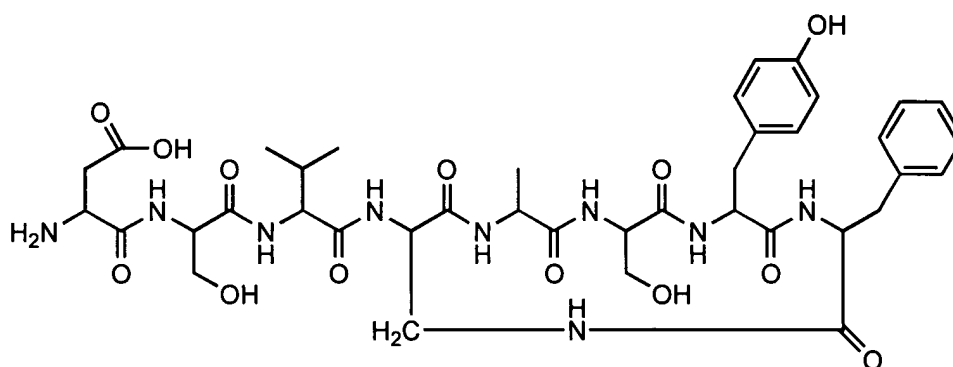
60. The antibiotic composition of claim 52, wherein the inhibitor inhibits the peptide-mediated quorum-sensing pathway.

61. The antibiotic composition of claim 60, wherein the antibiotic is ampicillin.

62. The antibiotic composition of claim 60, wherein the inhibitor is of the structure:



or of the structure:



or of the structure:

(cyclo)-YSTCDFIM;  
(cyclo)-GVNACSSLF;  
(cyclo)-GVNASSSLF; or  
(cyclo)-GVNA(DAPA)SSLF,

wherein in the latter four structures the C-terminal carbonyl group forms a thiolactone with sulfur atom of the cysteine residue (YSTCDFIM AND GVNACSSLF);

a lactone group with the oxygen atom of the first serine residue (GVNASSSLF); or

an amide bond with amino group of the diaminopropionic acid (DAPA) residue (GVNA(DAPA)SSLF).

63. The antibiotic composition of claim 60, wherein the antibiotic is selected from the group consisting of a penicillin, a quinoline, vancomycin, and a sulfonamide.

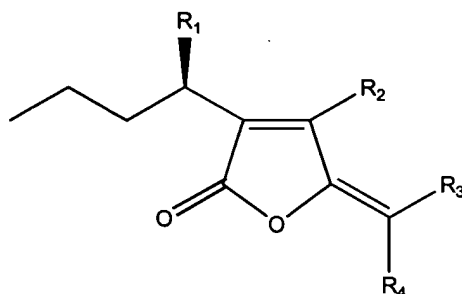


64. The antibiotic composition of claim 63, wherein the antibiotic is selected from the group consisting of ampicillin, ciprofloxacin, and sulfisoxazole.

65. A pharmaceutical composition comprising an antibiotic composition comprising an antibiotic and an inhibitor of a quorum-sensing pathway of a microorganism or a pharmaceutically-acceptable salt thereof and one or more pharmaceutically acceptable carriers, adjuvants or vehicles.

66. The composition of claim 65, wherein the quorum-sensing pathway is the AI-1 quorum-sensing pathway.

67. The composition of claim 66, wherein the inhibitor is a halogenated 2(5H)furanone of the structure:



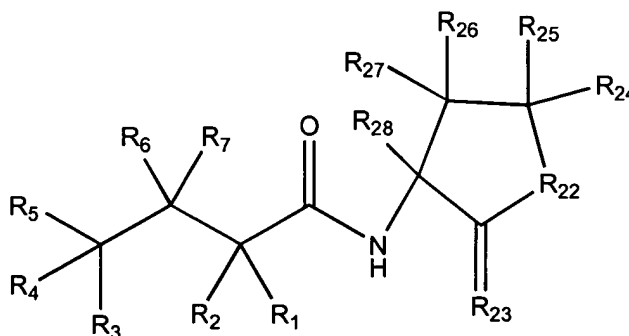
wherein R<sub>1</sub> is a hydrogen atom, an acetoxy group, or a hydroxy group;

R<sub>2</sub> is a hydrogen atom or a bromo group;

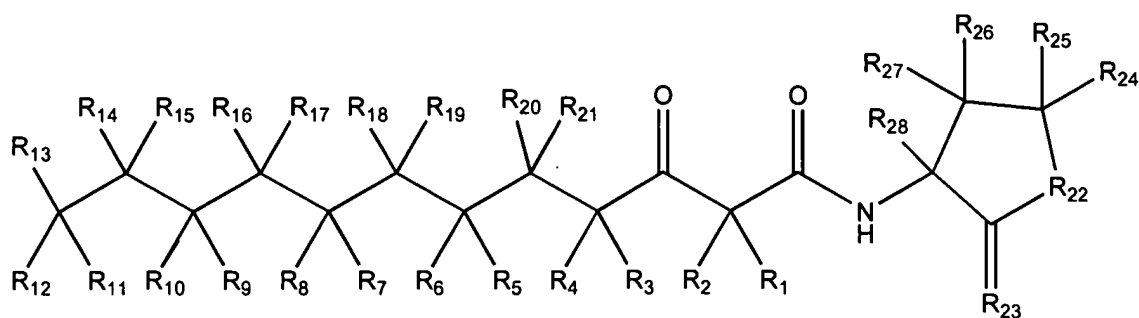
R<sub>3</sub> is a hydrogen atom or a bromo group; and

R<sub>4</sub> is a bromo or iodo group.

68. The composition of claim 66, wherein the AI-1 inhibitor is a modified N-butyryl-L-homoserine lactone compound of the structure:



or a modified N-(3-oxododecanoyl)-L-homoserine lactone compound of the structure:



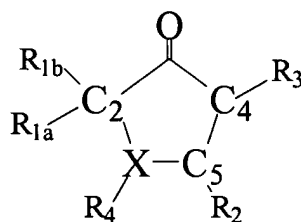
wherein in the above structures  $R_1$  through  $R_{21}$  are each individually selected from the group consisting of a  $C_1$  to  $C_4$  alkyl group, a hydrogen atom, a hydroxy group, an amino group, and a thiol group;

$R_{22}$  and  $R_{23}$  are each individually an oxygen or a sulfur atom; and

$R_{24}$  -  $R_{28}$  are each individually a hydrogen atom or a halogen atom.

69. The pharmaceutical composition of claim 65, wherein the inhibitor inhibits the AI-2 quorum-sensing pathway.

70. The pharmaceutical composition of claim 69, wherein the inhibitor that inhibits the AI-2 quorum-sensing pathway comprises the structure:



wherein X is oxygen, sulfur or nitrogen;

$R_{1a}$  is hydrogen, hydroxy, alkyl, acyl, amido, hydroxyl, amino, thio, or aryl;

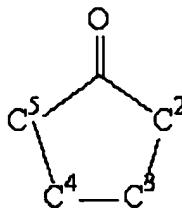
$R_{1b}$  is hydrogen, hydroxy, alkyl, acyl, amido, hydroxyl, amino, mercapto, thio, or aryl, or  $R_{1a}$  and  $R_{1b}$  together form a double bond;

$R_2$  is hydrogen, alkyl, or halogen;

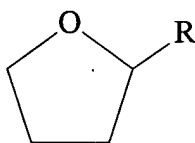
$R_3$  is hydrogen, alkyl, acyl, amido, hydroxyl, amino, thio, or aryl;

$R_4$  is hydrogen, if X is nitrogen, or is absent if X is oxygen or sulfur; and

wherein  $C_4$  and  $C_5$  can optionally be further joined by a double bond; or comprises the structure



wherein C<sup>2</sup> is additionally bonded to at least one substituent selected from hydrogen, hydroxyl, C<sub>1-5</sub> alkyl, C<sub>2-5</sub> alkenyl, C<sub>2-5</sub> alkanoyl, C<sub>2-5</sub> alkanoyloxy, heteroaryl, or forms a double bond with an oxygen atom or C<sup>3</sup>; wherein C<sup>3</sup> is additionally bonded to at least one substituent selected from hydrogen, hydroxyl, C<sub>1-5</sub> alkyl, C<sub>2-5</sub> alkenyl, C<sub>2-5</sub> alkanoyl, C<sub>2-5</sub> alkanoyloxy, or forms a double bond with an oxygen atom or C<sup>2</sup>; wherein C<sup>4</sup> is additionally bonded to at least one substituent selected from hydrogen, C<sub>1-5</sub> alkyl, C<sub>2-5</sub> alkanoyl, C<sub>2-5</sub> alkanoyloxy, or forms a double bond with an oxygen atom or C<sup>5</sup>; and wherein C<sup>5</sup> is additionally bonded to at least one substituent selected from hydrogen, C<sub>1-5</sub> alkyl, C<sub>2-5</sub> alkanoyl, C<sub>2-5</sub> alkanoyloxy, or forms a double bond with an oxygen atom or C<sup>4</sup>, wherein at least one of C<sup>2</sup>, C<sup>3</sup>, C<sup>4</sup> or C<sup>5</sup> is bonded to a substituent selected from hydroxyl, C<sub>1-5</sub> alkyl, C<sub>2-5</sub> alkenyl, C<sub>2-5</sub> alkanoyl, and heteroaryl, and wherein at most one carbon-carbon double-bond is present in the ring; or comprises the structure



wherein R is a C<sub>1-5</sub> alkoxy group.

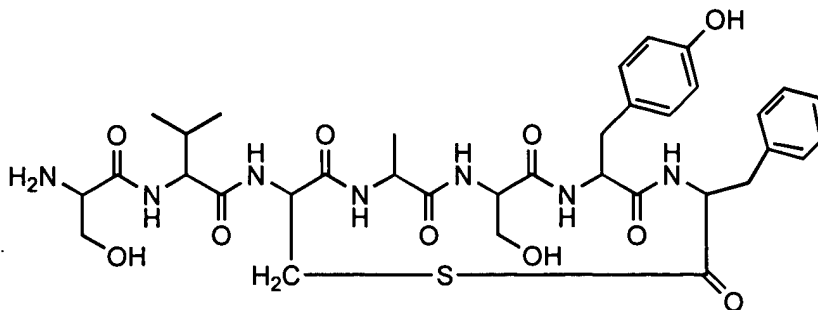
71. The pharmaceutical composition of claim 70, wherein the inhibitor is 5-methyl-2-ethyl-4-hydroxy-3(2H)-furanone.

72. The pharmaceutical composition of claim 70, wherein the inhibitor is 2,5-dimethyl-4-hydroxy-3(2H)-furanone.

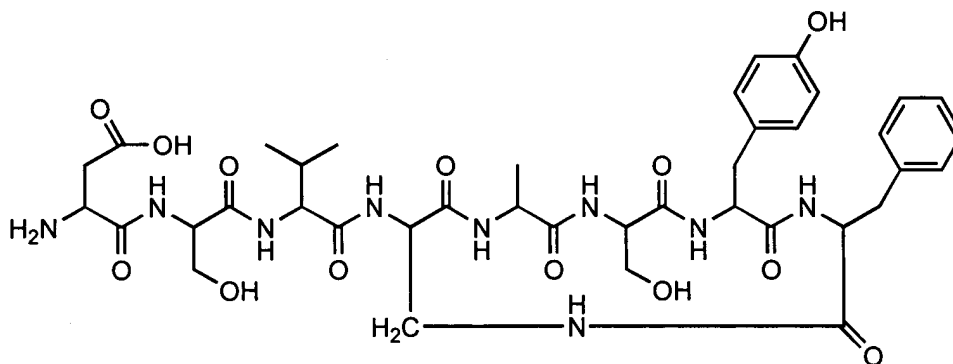
73. The pharmaceutical composition of claim 70, wherein the inhibitor is 2-methoxy-2,4-diphenyl-3(2H)-furanone.

74. The pharmaceutical composition of claim 65, wherein the inhibitor inhibits the peptide-mediated quorum-sensing pathway.

75. The pharmaceutical composition of claim 74, wherein the inhibitor is of the structure:



or of the structure:



or of the formula:

(cyclo)- YSTCDFIM;  
(cyclo)- GVNACSSLF;  
(cyclo)- GVNASSSLF; or  
(cyclo)- GVNA(DAPA)SSLF,

wherein in the latter four formulas the C-terminal carbonyl group forms a thiolactone with the sulfur atom of the cysteine residue (YSTCDFIM and GVNACSSLF);

a lactone group with the oxygen atom of the first serine residue (GVNASSSLF); or

an amide bond with amino group of the diaminopropionic acid (DAPA) residue (GVNA(DAPA)SSLF).

76. The pharmaceutical composition of claim 74, wherein the antibiotic is selected from the group consisting of a penicillin, a quinoline, vancomycin, and a sulfonamide.

77. The pharmaceutical composition of claim 76, wherein the antibiotic is selected from the group consisting of ampicillin, ciprofloxacin, and sulfisoxazole.

78. The antibiotic composition of claim 52, wherein the antibiotic composition is a synergistic antibiotic composition.

79. The pharmaceutical composition of claim 65, wherein the antibiotic composition is a synergistic antibiotic composition.

80. A method of treating infections in a warm-blooded animal caused by microorganisms possessing a quorum-sensing mechanism, comprising administering to the animal a therapeutically effective amount of an antibiotic composition comprising an antibiotic and an inhibitor of the quorum-sensing pathway of a microorganism.

81. The method of claim 80, wherein the microorganism is *Streptococcus pyogenes*.

82. The method of claim 81, wherein the antibiotic is selected from the group consisting of a penicillin, a quinoline, vancomycin, and a sulfonamide.

83. The method of claim 82, wherein the antibiotic is selected from the group consisting of ampicillin, ciprofloxacin, and sulfisoxazole.

84. The method of claim 80, wherein the microorganism is *Staphylococcus aureus*.

85. The method of claim 84, wherein the antibiotic is ampicillin.

86. The method of claim 84, wherein the inhibitor inhibits the AI-2 quorum sensing pathway.

87. The method of claim 80, wherein the antibiotic composition is a synergistic antibiotic composition.

88. The method of claim 80, wherein the warm-blooded animal is a human.

89. A method of treating infections in a warm-blooded animal caused by microorganisms possessing a quorum-sensing mechanism, comprising administering to the animal a therapeutically effective amount of a pharmaceutical composition, wherein the pharmaceutical composition comprises an antibiotic composition comprising an antibiotic and an inhibitor of a quorum-sensing pathway of a microorganism or a pharmaceutically-

acceptable salt thereof and one or more pharmaceutically acceptable carriers, adjuvants or vehicles.

90. The method of claim 89, wherein the antibiotic is selected from the group consisting of a penicillin, a quinoline, vancomycin, and a sulfonamide.

91. The method of claim 90, wherein the antibiotic is selected from the group consisting of ampicillin, ciprofloxacin, and sulfisoxazole.

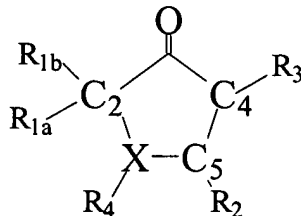
92. The method of claim 89, wherein the microorganism is *Streptococcus pyogenes* or *Staphylococcus aureus*.

93. The method of claim 92, wherein the antibiotic is ampicillin.

94. The method of claim 89, wherein the inhibitor inhibits the AI-2 quorum sensing pathway.

95. The method of claim 89, wherein the antibiotic composition is a synergistic antibiotic composition.

96. A medical device comprising at least one antimicrobial compound comprising the structure:



wherein X is oxygen, sulfur or nitrogen;

R<sub>1a</sub> is hydrogen, hydroxy, alkyl, acyl, amido, hydroxyl, amino, thio, or aryl;

R<sub>1b</sub> is hydrogen, hydroxy, alkyl, acyl, amido, hydroxyl, amino, mercapto, thio, or aryl, or R<sub>1a</sub> and R<sub>1b</sub> can together form a double bond;

R<sub>2</sub> is hydrogen, alkyl, or halogen;

R<sub>3</sub> is hydrogen, alkyl, acyl, amido, hydroxyl, amino, thio, or aryl;

R<sub>4</sub> is hydrogen, if X is nitrogen, or is absent if X is oxygen or sulfur; and

wherein C<sub>4</sub> and C<sub>5</sub> can optionally be further joined by a double bond, wherein the compound is present in a concentration sufficient to provide a localized antimicrobial effect.

97. A medical device comprising at least one synergistic antibiotic composition as set forth in claim 78, wherein the composition is present in a concentration sufficient to provide a localized antimicrobial effect.

98. A medical device comprising at least one pharmaceutical composition as set forth in claim 65, wherein the composition is present in a concentration sufficient to provide a localized anti-microbial effect.